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Lüscher, Thomas F ; Landmesser, Ulf ; von Eckardstein, Arnold ; Fogelman, Alan M

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This Review is part of a thematic series on **High-Density Lipoprotein**, which includes the following articles:

Mendelian Disorders of High Density Lipoprotein Metabolism [*Circ Res*. 2014;114:124–142]
Regulation of High-Density Lipoprotein Metabolism [*Circ Res*. 2014;114:143–156]
ATP-Binding Cassette Transporters, Atherosclerosis, and Inflammation [*Circ Res*. 2014;114:157–170]
High-Density Lipoprotein: Vascular Protective Effects, Dysfunction, and Potential as Therapeutic Target
MicroRNA Control of High-Density Lipoprotein Metabolism and Function
Novel Therapies Focused on the High-Density Lipoprotein Particle
High-Density Lipoprotein and Atherosclerosis Regression: Evidence From Preclinical and Clinical Studies
Population Genetics
Cholesterol Efflux and Reverse Cholesterol Transport
Proteomics and Particles

Alan Tall, Daniel Rader, Editors

High-Density Lipoprotein

Vascular Protective Effects, Dysfunction, and Potential as Therapeutic Target

Thomas F. Lüscher, Ulf Landmesser, Arnold von Eckardstein, Alan M. Fogelman

Abstract: **High-density lipoprotein (HDL) is a complex mixture of lipoproteins that is associated with many minor proteins and lipids that influence the function of HDL. Although HDL is a promising marker and potential therapeutic target based on its epidemiological data and the effects of healthy HDL in vitro in endothelial cells and macrophages, as well as based on infusion studies of reconstituted HDL in patients with hypercholesterolemia, it remains still uncertain whether or not HDL cholesterol–raising drugs will improve outcomes. Recent studies suggest that HDL becomes modified in patients with coronary artery disease or acute coronary syndrome because of oxidative processes that result in alterations in its proteome composition (proteome remodelling) leading to HDL dysfunction. (*Circ Res*. 2014;114:171-182.)**

Key Words: apolipoprotein A-I ■ cholesterol, HDL ■ nitric oxide

In population studies, high-density lipoprotein cholesterol (HDL-C) is inversely related to the risk of myocardial infarction and death.[1–4] Of note, in patients fully treated according to current guidelines with intense statin therapy and low-density lipoprotein cholesterol (LDL-C) at target levels, HDL-C remains predictive of outcome for major adverse cardiovascular events.[5] Accordingly, the metabolism and the vascular effects of HDL have attracted enormous interest. Furthermore, HDL is being intensely investigated as a potential therapeutic target in patients at high cardiovascular risk. In line with a causally protective effect, HDL can exert a broad spectrum of antiatherogenic effects capable of halting or even reversing atherosclerosis in several animal models, in particular by transgenic overexpression or exogenous application of apolipoprotein A-I (apoA-I), the most abundant protein of HDL.[6]

Unfortunately, it has been proven difficult to reduce coronary risk with drugs increasing HDL-C, such as fibrates, niacin, or inhibitors of cholesteryl ester transfer protein (CETP), beyond that achieved with statin therapy alone.[7–10] Moreover, in several inborn errors of human HDL metabolism and genetic mouse models with altered HDL metabolism, the changes in HDL-C levels were not associated with accompanying changes in cardiovascular risk or atherosclerotic plaque load, respectively, as has been expected from epidemiological studies.[11–13] Thus, the pathogenic role and, hence, suitability of HDL as a therapeutic target has increasingly been questioned.

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Nonstandard Abbreviations and Acronyms	
ABCA1	ATP-binding cassette transporter A1
ABCG1	ATP-binding cassette transporter G1
ACS	acute coronary syndrome
apoA-I	apolipoprotein A-I
CAD	coronary artery disease
CETP	cholesteryl ester transfer protein
eNOS	endothelial NO synthase
HDL-C	high-density lipoprotein cholesterol
LCAT	lecithin/cholesterol acyltransferase
LDL-C	low-density lipoprotein cholesterol
RCT	reverse cholesterol transport
S1P	sphingosine-1-phosphate
SR-BI	scavenger receptor BI
VCAM1	vascular cell adhesion molecule 1

In fact, it has been argued that low HDL-C may only represent a marker for proatherogenic risk factors, rather than HDL being a mediator protecting against atherogenesis. However, although not disputed, such a conclusion neglects the structural and functional heterogeneity of HDL, which is neither reflected by the nonfunctional clinical biomarker HDL-C nor has yet been targeted by randomized controlled trials[7–10] or Mendelian randomization studies.[13,14]

Structure of HDL

In a prototypic HDL particle, 2 to 5 molecules of apoA-I and ≈100 molecules of phosphatidylcholine form an amphipathic shell in which several molecules of unesterified cholesterol are imbedded surrounding a core of completely water-insoluble cholesterol esters.[15] Molar differences in the content of major proteins and lipid constituents of HDL, that is apoA-I, phosphatidylcholine, sphingomyelin, cholesterol, and cholesteryl esters, cause considerable heterogeneity of HDL as delineated by electron microscopy (shape), ultracentrifugation (density), gel filtration, polyacrylamide gel electrophoresis, nuclear magnetic resonance spectroscopy (size), charge (agarose gel electrophoresis), or affinity (apolipoprotein composition).[16] Unfortunately, there is only limited overlap between the proposed HDL subclasses defined by the various isolation methods. To facilitate communication and application in clinical studies, a consensus group has suggested 5 HDL subclasses according to size (small, very small, medium, large, very large).[16] To date, clinical and epidemiological studies have come to discrepant conclusions on the prognostic performance of HDL subclasses.[16–18]

This macroheterogeneity is further increased by different quantities of proteins or lipids, some of which may contribute to the pleiotropic functions of HDL. HDL particles carry >80 different proteins[19–21] and hundreds of lipid species.[19,22] Most recently, even microRNAs have been found in HDL particles,[23] although a relevant uptake of microRNAs from HDL into vascular cells was not observed.[24] Many of these molecules are not passive cargo but biologically active and contribute to pleiotropic and antiatherogenic properties of HDL. Of note, these molecules are not only involved in lipid transport and metabolism (ie, apoA-I, apoA-II), but also in oxidation or antioxidation (eg, paraoxonase), innate immune defense (eg, lipopolysaccharide-binding protein, clusterin, apoL1), or regulation of cell survival, proliferation, and migration (eg, sphingosine-1-phosphate [S1P], clusterin). Importantly, the plasma concentration of many microcomponents is by 1 or 2 orders of magnitude lower than that of HDL particles (10–20 μmol/L). Accordingly, they are not equally distributed among HDL subclasses. Interestingly, such microcomponents seem to accumulate in the smallest HDL particles (ie, HDL_3).[20] At first sight, this is in contrast to their small size. However, HDL_3 particles are present in plasma at the highest concentration, which is considerably higher than that of its microcomponents, suggesting that they are not residing on the same but on different particles.

Of note, this microheterogeneity of HDL particles of healthy subjects is even more pronounced in patients with inflammatory diseases, diabetes mellitus, coronary artery disease (CAD), or chronic kidney disease because of loss or structural modification of HDL constituents or by the acquisition of atypical constituents.[25–31] Several of these changes have been associated with a loss of vasoprotective functions, such as loss of the ability to stimulate cholesterol efflux after chlorination or nitration of tyrosine residues in apoA-I,[32] loss of the ability to inhibit endothelial apoptosis,[28] or loss of the ability to induce endothelium-dependent vasodilation after lipid peroxidation.[26] HDL may even acquire properties adverse to endothelial function, for example, by accumulation of malondialdehyde or symmetrical dimethylarginine.[27,29] Symmetrical dimethylarginine was shown to modify HDL such that it is recognized by toll-like receptor-2, leading to inhibition of endothelial NO synthase (eNOS)-activating pathways, stimulation of NADPH oxidase, impaired endothelial repair, increased proinflammatory activation, and increased arterial blood pressure.[29]

The very discrepant concentrations of HDL-C (>1 mmol/L), compared with apoA-I (≈50 μmol/L), or minor bioactive components of HDL (eg, PON1 or clusterin, 1 μmol/L; S1P, 0.5 μmol/L; apoL1, 0.1 μmol/L), or modifications of HDL (micromoles per liter or less), would predict the lack of sensitivity and specificity of plasma levels of HDL-C and apoA-I to reflect changes in structure–function relationships of HDL in healthy subjects and patients with cardiovascular disease.

Thus, depending on the mode of action, therapeutic interventions in HDL metabolism will either reduce or further increase the structural heterogeneity of HDL beyond what can be measured by conventional HDL-C or apoA-I assays. Functional HDL high-throughput assays that assess structure–function relationships of HDL in health and disease as well as after therapeutic interventions need to be developed (eg, multiparametric measurements of HDL-associated proteins by single-reaction monitoring mass spectrometry or arrayed immunoassays and delineation of lipid species). The ability to make such measurements could lead to the discovery of components that are more closely associated with cardiovascular risk and treatment effects, compared with plasma concentrations of HDL-C and apoA-I.

Metabolism of HDL

The majority of HDL particles originate from lipid-free or poorly lipidated apoA-I that is secreted by hepatocytes and the intestinal mucosa, or dissociate from lipolysed chylomicrons

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and very low-density lipoproteins as well as from interconverting mature HDL particles.[11,33] The interaction of lipid-free or poorly lipidated apoA-I, the so-called pre-β_1-HDL, with the ATP-binding cassette transporter A1 (ABCA1) leads to efflux of phospholipids and unesterified cholesterol from many cells, including hepatocytes, enterocytes, and macrophages, and to the formation of small discoidal HDL particles, called α_4-HDL. These HDL precursors can continue to induce lipid efflux from cells, for example, via scavenger receptor BI (SR-BI) or ATP-binding cassette transporter G1 (ABCG1).[33] The effluxed cholesterol and phosphatidylcholine serve as substrates of lecithin/cholesterol acyltransferase (LCAT) to form water-insoluble cholesteryl esters, which form the core of mature spherical HDL. These initially small α-HDL$_3$ particles become larger α-HDL$_2$ particles through ongoing acquisition of phospholipids and cholesterol from both cells (eg, via interactions with SR-BI or ABCG1) and apoB-containing lipoproteins and fusion with other HDL particles. The latter 2 processes involve the activity of phospholipid transfer protein.[11,33] The catabolism of HDL differs from that of LDL because only a minor proportion of HDL seems to be eliminated by holoparticle uptake into cells. In this as-yet-poorly-understood pathway, a high-affinity interaction of apoA-I with ectopic F0F1–ATPase leads to the formation of ADP, which activates purinergic receptors to induce the uptake of HDL by an as-yet-unidentified low-affinity HDL receptor.[34–36]

The 2 better-characterized pathways mediate the removal of lipids from HDL independently of its protein moiety: CETP exchanges triglycerides of apoB-containing lipoproteins for cholesteryl esters of HDL, which are ultimately eliminated via the LDL receptor pathway, and SR-BI mediates the selective uptake of HDL lipids into the liver and steroidogenic organs.[11,33] The removal of cholesteryl esters by CETP and SR-BI, as well as the lipolysis of triglycerides and phospholipids by hepatic lipase and endothelial lipase, respectively, leads to the conversion of HDL$_2$ into HDL$_3$ as well as the generation of pre-β_1-HDL.[11,33] This lipid-free or lipid-poor apoA-I either regenerates mature HDL by inducing ABCA1-mediated lipid efflux from various cells, or is filtrated through the renal glomeruli. In the proximal tubule of the kidneys, apoA-I is endocytosed by the cubilin and megalin receptors and targeted for lysosomal degradation.[37]

Cholesterol Efflux and Reverse Cholesterol Transport

Reverse cholesterol transport (RCT)[33] describes the transport of excess cholesterol from peripheral cells to the liver for excretion into the bile, either directly or indirectly after conversion into bile acids, or to adrenals, testes, and ovaries for steroid hormone production. In the classical model, HDL particles are considered passive acceptors of cellular cholesterol, in which LCAT-mediated esterification prevents equilibration of cholesterol between cell membranes and HDL, thereby allowing net cholesterol efflux.[38] Because of the RCT model, it is still erroneously thought that HDL-C levels reflect the amount of cholesterol released from peripheral cells and transported by HDL back to the liver. The discovery of several rate-limiting genes and the metabolic characterization of patients and genetic mouse models that lack these limiting factors have led to significant revisions of the RCT model and ruled out that HDL-C levels reflect the activity of RCT.[33]

In addition to Glomset aqueous diffusion, cholesterol efflux is facilitated by tethering of HDL to SR-BI and ABCA1- or ABCG1-facilitated transport to mediate the active export of excess cholesterol to α-HDL (SR-BI and ABCG1) and lipid-poor apoA-I (ABCA1).[33] The transporters ABCA1 and ABCG1 are integrated into both positive feed-forward and negative feedback regulation loops. Cellular lipid overload leads to the formation of oxysterols, which activate nuclear liver X receptors α and β to induce the transcription of ABCA1 and ABCG1 and hence cholesterol efflux.[39] It is presently not clear how ABCG1 facilitates sterol transport to acceptors at the cell surface, because it was reported to be an intracellular transporter.[40] Nonetheless, there is no controversy about its role in promoting cholesterol efflux.[33] Several microRNAs, for example, miR33, miR122, and miR144, decrease the production of ABCA1 and hence reduce cholesterol efflux.[41–43] Thus, cholesterol efflux is determined by the extracellular concentration and composition of HDL particles as well as by the activity of ABC transporters. By finetuning cellular cholesterol homeostasis, cholesterol efflux by apoA-I/ABCA1- and HDL-mediated efflux in concert with ABCG1 exerts important regulatory steps on many cellular functions, including proliferation and mobilization of hematopoietic stem cells.[43] ATP-binding cassette transporter G4–mediated cholesterol efflux to HDL regulates megakaryocyte proliferation.[44] Cholesterol efflux also regulates the inflammatory responses of monocytes and macrophages,[45] expansion of lymphocytes,[46] NO production by eNOS,[47] and insulin secretion from pancreatic β-cells.[48] To test the relevance of cholesterol efflux in clinical studies, bioassays have been developed to quantify cellular cholesterol efflux activity of fibroblasts or monocytes, cholesterol acceptor capacity of HDL, and total or apoB-depleted serum or plasma from patients. Studies in families with inborn errors of HDL metabolism confirmed the feasibility of these bioassays; that is, cholesterol efflux in total or apoB-depleted plasmas from patients with apoA-I, LCAT, or ABCA1 deficiency is strongly reduced and cholesterol efflux from fibroblasts and monocytes carrying mutations in ABCA1 is decreased in a gene dosage–dependent fashion.[49,50] Measurements of cholesterol efflux of total or apoB-depleted plasmas have been used in clinical and epidemiological studies, however with conflicting results.[51] Characterization of interferences and disturbances by the sample specimens used, as well as standardization of preanalytical and analytic procedures, is needed for further exploitation of cholesterol efflux capacity as a biomarker in drug development.[51]

Despite the pivotal role of apoA-I and ABCA1 for cholesterol efflux and HDL formation, mice deficient of ABCA1 or apoA-I excrete normal amounts of sterols in their feces with no disturbances in body cholesterol homeostasis.[52] Moreover, both transplantation of ABCA1-deficient bone marrow cells and targeted knockout of ABCA1 in macrophages in mice showed that cholesterol efflux from macrophages does not directly affect HDL-C plasma levels.[53,54] Such incompatibilities with Glomset RCT model were partially resolved by the concept of macrophage-specific RCT and its validation

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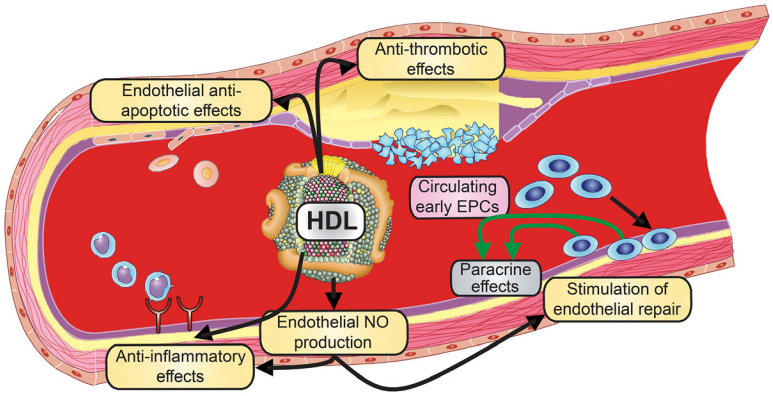

Figure 1. Proposed direct vascular protective and potentially antiatherogenic effects of normal high-density lipoprotein (HDL).

in a novel experimental animal model: peritoneal or bone marrow–derived macrophages loaded with radioactive cholesterol were installed into the peritoneum of recipient mice to record the uptake of radioactive cholesterol into the plasma and liver as well as its excretion into feces.[55,56] Fecal excretion of macrophage-derived radioactive sterols was found to be decreased by the knockout of ABCA1 or apoA-I and increased by the overexpression of apoA-I. By applying this experimental procedure to genetic animal models, the importance of several rate-limiting genes in RCT was verified (ie, apoA-I, ABCA1, ABCG1, SR-BI, or CETP), but also refuted (LCAT).[55,56] Novel bottlenecks of RCT (eg, intestinal cholesterol resorption by NPC1L1) were identified using this model.[57] It also contributed to the growing evidence for the existence of a liver- and HDL-independent RCT pathway, the so-called transintestinal cholesterol excretion.[58] Notably, compared with HDL-C levels, the activity of macrophage-specific RCT also showed stronger correlations with atherosclerosis in different animal models with disturbed HDL metabolism.[56] Unfortunately, macrophage-specific RCT in humans could not be assessed as yet. In clinical studies, measurement of fecal sterol excretion or the recently described isotopic dilution method does not distinguish macrophage-specific RCT from general RCT.[59]

Vascular Protective Effects of HDL

HDL from healthy subjects can exert several protective effects in the vasculature and, in particular, on endothelial cells (Figure 1). Of note, HDL from healthy subjects stimulates NO release from human aortic endothelial cells in culture (Figure 2) and increases the expression of eNOS.[27,60–62] Furthermore, it suppresses the expression of adhesion molecules, such as vascular cell adhesion molecule 1, and inhibits the adhesion of white blood cells.[25,63] HDL also exerts antithrombotic effects because it reduces tissue factor expression in endothelial cells exposed to cytokines and reduces platelet activation.[64] Furthermore, in the mouse carotid artery model, HDL enhances endothelial repair after vascular injury.[27] Finally, HDL obtained from healthy subjects induced expression of Bcl-xL, the endothelial antiapoptotic Bcl-2 protein, and reduced endothelial cell apoptosis in vitro and in apoE-deficient mice in vivo.[28]

Such effects are seen not only in vitro and in animal models in vivo, but also in patients with mutations in ABCA1.[65] In patients with hypercholesterolemia, infusion of reconstituted HDL particles consisting mainly of apoA-I and phospholipids (and thus resembling pre-β_1HDL)[66] almost doubles HDL-C plasma levels and improves impaired endothelial function as assessed by intra-arterial infusion of acetylcholine and measurement of forearm blood flow by plethysmography or high-resolution ultrasound of the brachial artery and flow-mediated vasodilation, respectively (Figure 3).[66] Furthermore, in patients with CAD, weekly intravenous infusions of apoA-1_{Milano}, a genetic variant of apoA-I with maintained or even improved functionality,[67] at dosages of 15 or 45 mg/kg body weight reduced coronary plaque volume as assessed by intravascular ultrasound.[68] It is to be noted that no dose–response relationship was notable in this pilot study. Infusion of ApoA-I neither ameliorated endothelial function in patients with acute coronary syndrome (ACS)[69] nor affected the plaques.[70]

Several agonists and receptors in HDL and endothelial cells, respectively, have been identified that are thought to mediate the vasoprotective effects of HDL; both NO-dependent and NO-independent mechanisms have been described. HDL was shown to activate eNOS by regulating the cholesterol homeostasis of caveolae, where eNOS is localized. In this respect, ABCG1 was identified as an important cellular mediator of these effects by facilitating efflux of cholesterol and

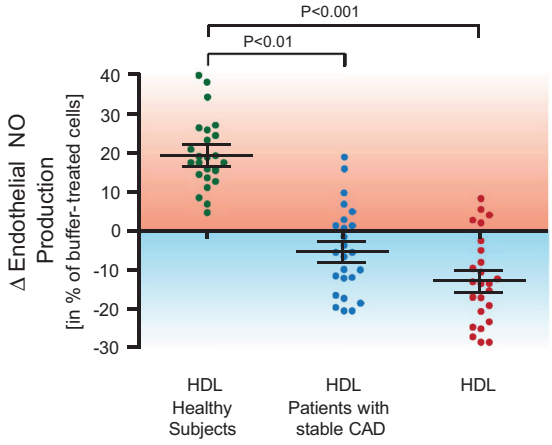

Figure 2. Effects of high-density lipoprotein obtained from healthy subjects, patients with coronary artery disease (CAD) or acute coronary syndrome on NO release from human aortic endothelial cells. Modified and adapted from Besler et al.[27] Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

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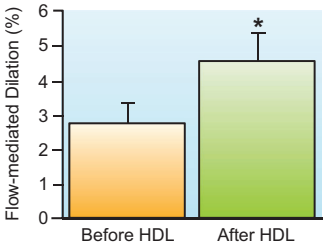

Figure 3. High-density lipoprotein (HDL) infusion improves endothelial function in humans. Acetylcholine-induced forearm blood flow in subjects with normal plasma cholesterol levels and in hypercholesterolemic subjects before and after infusion with reconstituted HDL. Modified and adapted from Spieker et al.[66] Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

7-ketocholesterol.[47] SR-BI was also found to be important for sensing the cholesterol concentration in plasma membrane domains of endothelial cells and enterocytes, independent of its ability to bind HDL or mediate cholesterol efflux.[71] Other laboratories provided evidence both in vitro and in vivo that S1P carried by HDL induces eNOS phosphorylation by binding to S1P1 and S1P3 receptors and thereby activating PI3 kinase and Akt phosphorylation. In fact, mice lacking either S1P3 or the S1P-binding protein apoM and thereby S1P showed reduced NO production as well as NO-dependent vasodilation and junction closure.[72,73] Several agonists and receptors have also been identified, which promote endothelial integrity by eNOS-independent mechanisms. S1P associated with HDL was found to stimulate endothelial junction closure and to inhibit apoptosis.[74,75] Interaction of HDL with SR-BI was found to stimulate proliferation and migration of endothelial cells by signaling through its PDZ domain to small G-proteins.[76] Interaction of apoA-I with ectopic β-ATPase leads to the generation of ADP, which in turn activates a purinergic receptor to stimulate endothelial cell proliferation.[34] Furthermore, clusterin is an antiapoptotic agonist carried by HDL, which stimulates Akt phosphorylation and thereby inhibits cell death.[28] It is not yet clear whether these mechanisms operate in a parallel or integrated manner. In agreement with the latter concept, it has been suggested that by tethering HDL on endothelial cell, SR-BI facilitates the interaction of S1P with its receptors,[77] raising the possibility that SR-BI–mediated effect on eNOS may, at least partially, be mediated by S1P. Alternatively, HDL may promote S1P efflux from endothelial cells, which is known to involve the ATP-binding cassette transporter C1 and which would explain why initially S1P-free reconstituted HDL particles are able to stimulate eNOS phosphorylation.[78]

HDL Dysfunction in Cardiovascular Patients

The vascular effects exerted by HDL obtained from patients with a range of cardiovascular conditions differ substantially from the properties of HDL obtained from healthy subjects, termed HDL dysfunction.[79] Unlike HDL from healthy subjects, HDL from patients with diabetes mellitus, CAD, ACS, or chronic renal dysfunction no longer simulates NO release from endothelial cells in culture, but rather tends to inhibit it (Figure 2).[26,27,29] Similarly, inhibition of endothelial vascular cell adhesion molecule 1 expression as well as the adhesion of white blood cells to activated endothelial cells observed in HDL from healthy subjects is markedly reduced or absent, similar to the endothelial antiapoptotic effects when HDL of patients with stable CAD, or of patients with ACS, are examined.[25,27,28]

One of the molecular mechanisms of this so-called HDL dysfunction in patients with CAD is a reduced activity (in the presence of an increased protein level) of HDL-associated paraoxanase-1 (PON-1), which normally prevents HDL from oxidative modification (Figure 4). A reduced HDL-associated activity of PON-1 leads to the generation of modified HDL with endothelial PKCβ_2-activating properties, at least in part because of increased formation of malondialdehyde.[27] In contrast to HDL from healthy subjects, HDL from patients with CAD gains access to endothelial lectin-like oxidized LDL receptor-1 and activates PKCβ_2, which in turn leads to the inhibition of eNOS activation because of its phosphorylation at the inhibitory residue T495 rather than at its activating residue S1177. This eventually contributes to a loss of anti-inflammatory effects and endothelial repair capability of HDL.

Similarly, HDL from patients with CAD or ACS does not inhibit endothelial apoptosis because it fails to activate endothelial Bcl-xL, but stimulates endothelial proapoptotic pathways, in particular, p38-mitogen-activated protein kinase–mediated activation of the proapoptotic Bcl-2 protein tBid.[28] Because the antiapoptotic effects of healthy HDL in endothelial cells persisted after inhibition of eNOS and after delipidation and were not fully mimicked by apoA-I or reconstituted HDL, the HDL proteome may be importantly involved in the endothelial effects of HDL. HDL proteomic analyses and subsequent validations and functional characterizations suggest reduced

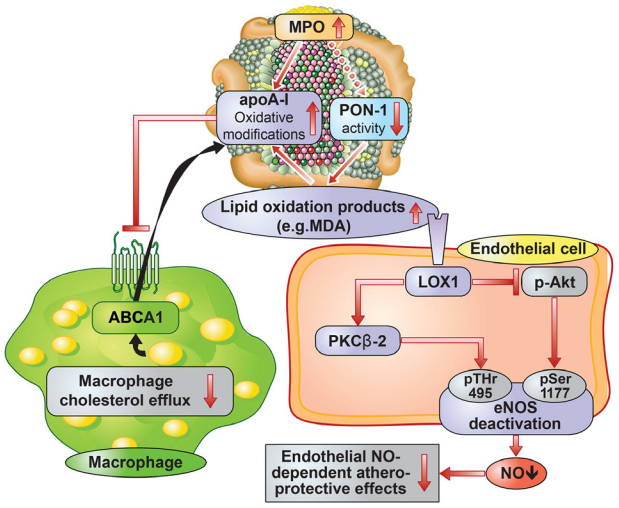

Figure 4. Mechanisms of altered vascular effects of high-density lipoprotein (HDL) in coronary disease. Activity of paraoxonase-1 (PON-1) is reduced in HDL obtained from patients with coronary artery disease. ABCA1 indicates ATP-binding cassette transporter A1; apoA-I, apolipoprotein A-I; eNOS, endothelial NO synthase; LOX1, lectin-like oxidized LDL receptor-1; MDA, malondialdehyde; and MPO, myeloperoxidase. Modified and adapted from Mineo et al.[114] Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

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clusterin and increased apo C-III content in HDL-CAD or HDL-ACS to be potential mechanisms (Figure 5).[28]

HDL as a Therapeutic Target

Based on the inverse epidemiological relation of HDL-C plasma levels with major adverse cardiovascular events, HDL-C has been considered an attractive therapeutic target. As a consequence, several drugs to raise HDL-C have been developed and investigated at basic and clinical levels. The first class of drugs that became available was the fibrates, which markedly lower plasma triglyceride levels and modestly increase HDL-C. Unfortunately, most trials with fibrates, particularly those performed recently, have yielded no or only minimal cardiovascular benefits with this class of drugs.[7,80]

Peroxisome proliferator-activated receptor-γ agonists such as rosiglitazone and pioglitazone are also able to increase HDL-C plasma levels in patients at risk or with CAD. Unfortunately, large outcome trials have yielded only minimal prognostic benefit with these drugs in this patient population.[81–84] Furthermore, it seemed that peroxisome proliferator-activated receptor-γ agonists increase the incidence of heart failure because of fluid retention.[85,86] Rosiglitazone has, therefore, been withdrawn from the market.[87] Pioglitazone has not been widely accepted by the cardiovascular community, and the US Food and Drug Admnistration has recently published a safety announcement related to its potential cancer risk.

Niacin is a hypolipidemic drug that has been used since the 1970s to treat patients with hyperlipidemia. Although its mechanism of action remains unclear,[88] niacin increases HDL-C by between 15% and 30% and lowers LDL-C and particularly triglycerides. The ARBITER 6-HALTS (Arterial Biology for the Investigation of the Treatment Effects of Reducing Cholesterol 6—HDL and LDL Treatment Strategies in Atherosclerosis) trial showed that niacin not only favorably modified lipid profiles, but also reduced plaque formation in carotid and coronary arteries.[89] Unfortunately, the large outcome trial AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides), supported by the National Institutes of Health, was stopped after a little more than 3000 patients had been recruited, because of futility.[9] The trial has been criticized for its small sample size and the fact that the dose of simvastatin (which was used as background therapy to control LDL-C) had been adapted to the LDL levels achieved with either placebo or niacin. Thus, patients on niacin received lower dosages of statin compared with patients on placebo, which can make the interpretation of the results challenging.

Big hopes rested on the Heart Protection Study 2—Treatment of HDL to Reduce the Incidence of Vascular Events (HPS-THRIVE) trial, which investigated the effect of extended-release niacin in combination with laropiprant (a prostaglandin D_2 receptor antagonist to reduce the incidence of flushing) in addition to simvastatin in 25 673 patients at high cardiovascular risk.[90,91] No significant benefit of the niacin–laropiprant combination on the primary outcome of major vascular events was seen when added to effective statin-based LDL-lowering therapy. Even worse, serious adverse events, such as diabetic complications and new onset diabetes mellitus as well as infections due to known and unrecognized

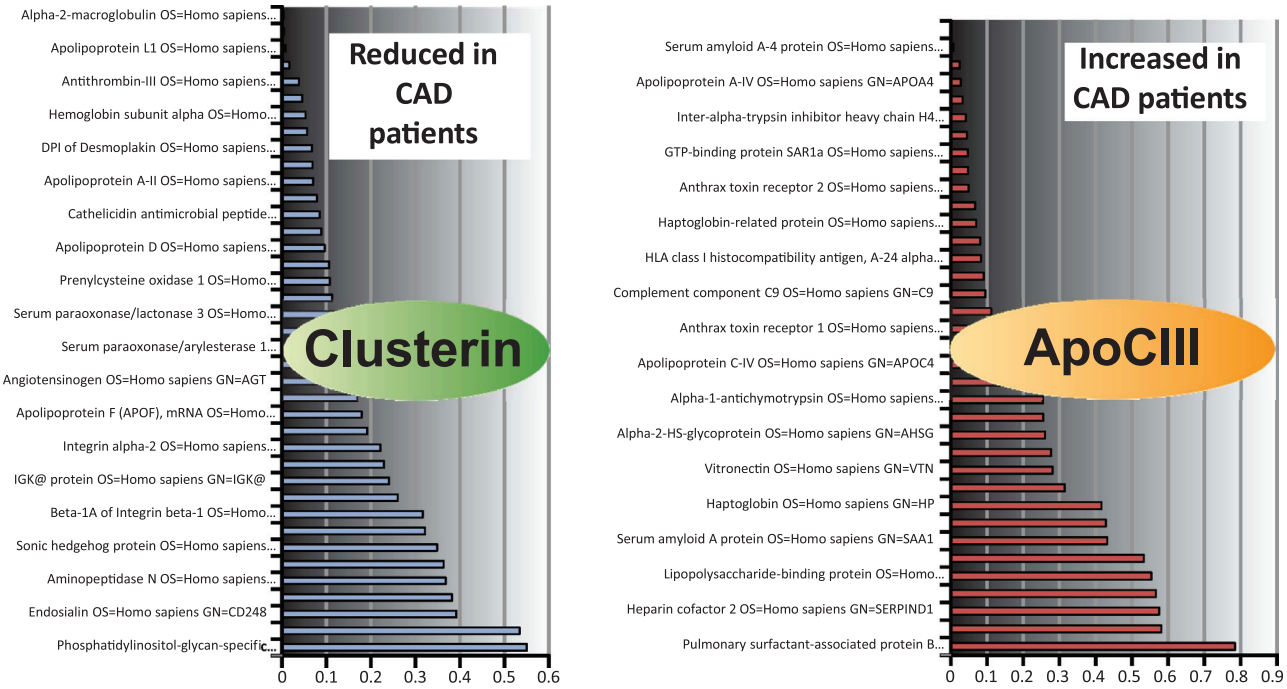

Figure 5. Proteins in high-density lipoprotein (HDL) from healthy subjects and patients with coronary artery disease (CAD) were identified and quantified using spectral index, and data are presented as proteins reduced or enriched in HDL-CAD. Gene ontology analysis suggested that changes in clusterin and apolipoprotein CIII are relevant for altered effects of HDL-CAD on endothelial apoptosis, which was also validated by further studies. Modified and adapted from Riwanto et al.[28] Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

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off-target effects of niacin (or laropiprant), became apparent. Moreover, the risk of myopathy was increased by adding the niacin–laropiprant combination to simvastatin 40 mg daily (with or without ezetimibe), particularly in Chinese patients whose myopathy rates on simvastatin were higher.[90]

Inhibition of CETP

The most potent novel class of HDL-C–increasing drugs is CETP inhibitors.[92] CETP is a plasma protein promoting the transfer of cholesterol esters from HDL to LDL (Figure 6). CETP inhibitors reduce the transfer of cholesterol esters in between different HDL particles, that is, from HDL_2 to HDL_3, and from HDL to very low-density lipoproteins or LDL, and thereby produce marked and consistent elevations of plasma HDL-C levels between 30% to 140%.[93] When added to a high dose of a statin, LDL-C either remains unchanged (for instance, with dalcetrapib) or decreases further ≈40% (with torcetrapib, anacetrapib, or evacetrapib).

In the large ILLUMINATE (Investigation of Lipid Level Management to Understand Its Impact in Atherosclerotic Events) trial involving 15 067 high-risk patients, the addition of torcetrapib to 80 mg of atorvastatin was found to be associated with increased mortality and morbidity.[8] This finding was particularly surprising because HDL-C increased by 80% and LDL-C decreased by 25% as compared with atorvastatin alone.[8] Interestingly, in the RADIANCE 2 (Rating Atherosclerotic Disease Change by Imaging with a New Cholesteryl-Ester-Transfer Protein Inhibitor) trial,[94] which used B-mode carotid ultrasound, as well as in the Investigation of Lipid Level Management Using Coronary Ultrasound to Assess Reduction of Atherosclerosis by CETP Inhibition and HDL Elevation (ILLUSTRATE) trial,[95] which used coronary intravascular ultrasound, torcetrapib did not reduce carotid intima-media thickness, nor did it decrease coronary plaque volume, despite favorable changes in the lipid profile. These unfavorable outcomes are likely related to off-target effects, such as increase in blood pressure and endothelial dysfunction. Although the blood pressure increase is likely related to increased aldosterone secretion from adrenal glands,[96,97] the vascular effects are due to the suppression of eNOS as well as increased release of reactive oxygen species and of endothelin-1.[98] Because such effects also occur in animals lacking CETP, such as rodents, they are likely off-target effects specific to this molecule.

Novel CETP Inhibitors

Subsequently, other CETP inhibitors such as anacetrapib, dalcetrapib, and evacetrapib have been developed, which seem to lack the off-target effects of torcetrapib. These compounds do not affect aldosterone secretion. In the Determining the Efficacy and Tolerability of CETP Inhibition with Anacetrapib (DEFINE) trial, anacetrapib was not found to raise blood pressure,[100] whereas in the dal-OUTCOMES trial, dalcetrapib was found to do so in a minor but statistically significant manner.[10] Furthermore, in the dal-VESSEL trial, dalcetrapib did not impair flow-mediated vasodilation, an index of endothelial function in humans in vivo.[99]

In the dal-PLAQUE study,[101] the effects of dalcetrapib on plaque formation and inflammation in the human carotid artery were investigated using MRI and PET technology. The results tended to show a small reduction in the increase in total vessel area, whereas inflammation as measured by fluorodeoxyglucose uptake remained unaffected. However, CRP levels were found to be increased by dalcetrapib treatment in the dal-OUTCOMES trial.[10] By contrast, CRP was unaffected by anacetrapib in DEFINE trial; in fact, it tended to be slightly higher in the treatment than in the placebo group.[100] As such, it is not surprising that the dal-HEART program was stopped by Roche on May 14, 2012, after an interim analysis

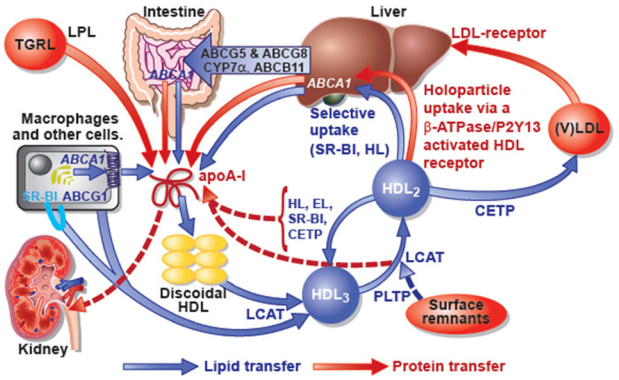

Figure 6. Schematic representation of high-density lipoprotein (HDL) metabolism. HDL metabolism is initiated by the secretion of lipid-free apolipoprotein (apoA-I) by the liver and intestine. Both hepatocytes and enterocytes express ATP-binding cassette (ABC) transporter A1 (ABCA1), which effluxes phospholipids and cholesterol and thereby lipidates apoA-I extracellularly. Lipid-free apoA-I is also generated by the lipolysis of triglyceride-rich lipoproteins (TGRL; chylomicrons and very low-density lipoprotein [VLDL]) and the interconversion of HDL subclasses in the course of lipolysis and lipid transfer and then interacts with ABCA1 in liver and peripheral cells. The resulting nascent HDL discs induce cholesterol efflux from macrophages by mechanisms involving ABCG1 and scavenger receptor BI (SR-BI). Lecithin/cholesterol acyltransferase (LCAT)-mediated cholesterol esterification turns the discoidal HDL into mature spherical particles. The initially smaller HDL_3 particles grow in size by ongoing lipid efflux, cholesterol esterification, and fusion with HDL and surface remnants of TGRL through phospholipid transfer protein (PLTP). The resulting HDL_2 particles deliver lipids to the liver, either directly via HDL receptors or indirectly via lipid transfer to LDL, which are then internalized by the LDL receptor pathway. In the direct pathway, SR-BI mediates the selective uptake of cholesteryl esters into hepatocytes. In addition to this nonendocytic pathway, an as-yet-unknown HDL receptor, which is stimulated by the interaction of apoA-I with ectopic β-ATPase and the subsequently activated P2Y13 receptor, mediates holoparticle uptake. The indirect pathway requires the exchange of cholesteryl esters and triglycerides between HDL and VLDL, which is mediated by the cholesteryl ester transfer protein (CETP). Reverse cholesterol transport is finalized by the biliary excretion of cholesterol from the liver into the intestine either directly via ABCG5 and ABCG8 or after cytochrome P enzyme (CYP7α)-mediated oxidation to bile acids via the bile salt export pump ABCB11. The actions of SR-BI, CETP, hepatic lipase (HL), and endothelial lipase (EL) on HDL_2, as well as of PLTP on HDL_3, lead to the interconversion of HDL particles and also liberate lipid-free apoA-I, which is either used for de novo formation of mature HDL particles or filtrated through the renal glomeruli for tubular uptake and degradation (red dotted arrows). Blue arrows indicate lipid transfers; and red arrows, protein or holoparticle tranfers. Modified and adapted from von Eckardstein.[11] Authorization for this adaptation has been obtained both from the owner of the copyright in the original work and from the owner of copyright in the translation or adaptation.

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of dal-OUTCOMES showed absolutely no benefit of dalcetrapib compared with placebo in patients after ACS.[10] Of note, the clinical outcome was precisely predicted by the results of the endothelial function study dal-VESSEL, suggesting that changes in flow-mediated vasodilation are a reliable surrogate end point in predicting treatment effects in high-risk patients.

The metabolic effects of the novel CETP inhibitors, however, differ: whereas dalcetrapib selectively increases HDL-C by only ≈30% and apoA-I by ≈18%, with LDL-C and ApoB-100 levels unaffected,[99] anacetrapib and evacetrapib have marked effects on LDL-C as well. The neutral effects of dalcetrapib may be related to the fact that a 30% increase in HDL-C may not be enough to alter endothelial function and clinical outcome in patients at risk. Moreover, HDL in patients with ACS may be dysfunctional and hence unable to exert protective effects when therapeutically raised.

In contrast, anacetrapib increases HDL-C by ≈140% and lowers LDL-C by 40% beyond the levels achieved with atorvastatin, as shown in the DEFINE trial[100]; this compound may improve outcome, hypothesizes the large REVEAL (Randomized Evaluation of the Effects of Anacetrapib Through Lipid Modification) trial, which is currently recruiting high-risk patients with a history of ACS, peripheral arterial or cerebrovascular disease, or diabetes mellitus.[102]

ApoA-I Mimetic Peptides

ApoA-I contains 243 amino acids. Peptides with only 18 amino acids that are not homologous to those in apoA-I, but which mimic the distribution of charge and structure of portions of apoA-I, were found to render HDL anti-inflammatory (as measured in an in vitro assay) and significantly reduce inflammation in several animal models of disease, including atherosclerosis.[103] Based on these preclinical studies, 2 small clinical trials were conducted. In the first trial, the apoA-I mimetic peptide was administered orally in doses from 0.43 to 7.14 mg/kg. The plasma levels achieved were very low (a maximum of ≈16 ng/mL). Peptide doses of 4.3 and 7.14 mg/kg significantly improved a measure of HDL functionality, the HDL inflammatory index; however, doses of 0.43 and 1.43 mg/kg did not.[104]

Because the therapeutic agent was an apoA-I mimetic peptide and the goal was to improve the function of a plasma lipoprotein (HDL), it was assumed that the peptide acted in the plasma, and plasma levels were a critical success factor. Therefore, the next trial targeted plasma peptide levels using low doses (0.042–1.43 mg/kg; doses that were ineffective in the first trial) but were administered intravenously or subcutaneously to achieve high plasma levels. High plasma levels were achieved (≈3250 ng/mL), but there was no improvement in the HDL inflammatory index.[105]

To understand these perplexing results, mice studies were performed. Surprisingly, when equal doses of peptide were administered, peptide levels were similar in the tissue of the small intestine, whether the peptide was administered orally or subcutaneously, but this was not the case in plasma or in liver where the plasma and liver levels, respectively, were 100- and 1000-fold higher after administering the peptide subcutaneously compared with orally. Efficacy as measured by the HDL inflammatory index, serum amyloid A levels, plasma lysophosphatidic acid levels, or by the extent of atherosclerotic lesions were identical at each dose regardless of the route of administration or the plasma peptide levels. At each dose, the concentration and amount of peptide in the feces were equal regardless of the route of administration or the plasma levels. It was concluded that (1) the dose of peptide administered, and not the plasma level, determined efficacy, and (2) the intestine is a major site of action for the peptide regardless of the route of administration.[106]

In a subsequent study, the administration of the same dose of peptide similarly reduced a large number of oxidized metabolites of arachidonic acid in small intestine enterocytes, whether the peptide was administered orally or subcutaneously, despite the 100- to 1000-fold greater liver and plasma levels of peptide after administration of the peptide subcutaneously compared with orally. These oxidized metabolites were significantly reduced to an even greater extent in liver and were not different whether peptide was administered subcutaneously or orally. Because the plasma and liver concentrations of peptide after administering the peptide orally were 100- to 1000-fold lesser compared with administering the peptide subcutaneously, these data strongly suggest that the reduction of oxidized metabolites of arachidonic acid in the small intestine by the peptide led to the reduction in liver levels.[107]

These results likely explain the failure of the clinical trial by Watson et al.[105] The dose administered was likely too low to achieve the needed peptide levels in the small intestine. The results also suggest that a dose of peptide on the order of 40 to 100 mg/kg per day will be required for efficacy. The 4F peptide used in the clinical trials requires chemical end groups so as to be efficient. These end groups can only be added by chemical synthesis, making 4F at such doses too costly for clinical use.[108,109] To overcome this problem, a search was conducted to find an apoA-I mimetic peptide that did not require these end groups for efficacy, and the peptide 6F was identified. The 6F peptide was transgenically expressed in tomatoes, freeze-dried, ground into powder, and added at 2.2% by weight to a Western diet to be fed to LDL receptor null mice.[109] The mice receiving transgenic 6F tomatoes had lower plasma levels of serum amyloid A, total cholesterol, and triglycerides compared with mice fed with control tomatoes. Levels of unsaturated lysophosphatidic acid were reduced in the tissue of the small intestine and in the plasma of mice fed with transgenic 6F tomatoes. Importantly, plasma HDL-C and PON-1 activity increased in the mice receiving tomatoes with 6F peptide, and aortic atherosclerosis reduced ≈50% compared with control mice. The levels of unsaturated lysophosphatidic acid in the tissue of the small intestine correlated with the percent of aorta with atherosclerotic lesions. Hours after the mice finished eating transgenic 6F tomatoes, intact peptide was found in the small intestine, but no peptide was found in the plasma, suggesting that the peptide was acting in the intestine and not in the plasma. It is tempting to speculate that the concentration of unsaturated lysophosphatidic acid and oxidized lipids in the small intestine is the target for apoA-I mimetic peptides, and the small intestine concentration of these lipids determines their concentration in liver and plasma.[106–109,111]

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The recent work of Navab et al[112,113] is consistent with this hypothesis. They found that feeding LDL receptor null mice a high-fat, high-cholesterol Western diet increased unsaturated (but not saturated) lysophosphatidic acid levels in the small intestine and caused changes in small intestine gene expression. They showed that the addition of transgenic tomatoes expressing the apoA-I mimetic peptide 6F to the Western diet prevented many Western diet–mediated small intestine changes in gene expression. If, instead of feeding the Western diet, unsaturated lysophosphatidic acid was added to the low-fat chow diet and fed to the mice, the levels of lysophosphatidic acid in the small intestine were similar to those induced by feeding the Western diet.[112] They found that gene expression changes in the small intestine after the addition of unsaturated lysophosphatidic acid to the low-fat chow diet mimicked the changes seen after feeding the Western diet and also mimicked the changes in plasma serum amyloid A, total cholesterol, triglycerides, HDL-C, and lipoprotein profiles seen after feeding the Western diet. Adding freeze-dried tomato powder from tomatoes transgenic for the 6F peptide (but not from control tomatoes) to the lysophosphatidic acid–supplemented low-fat chow diet prevented the lysophosphatidic acid–induced changes.[112] It was concluded that (1) Western diet–mediated systemic inflammation and dyslipidemia may be, in part, because of Western diet–induced increases in small intestine lysophosphatidic acid levels, and (2) transgenic tomatoes expressing the 6F peptide reduce Western diet–mediated systemic inflammation and dyslipidemia by preventing Western diet–induced increases in lysophosphatidic acid levels in the small intestine.[112]

These studies demonstrate the complexity of HDL and its proteins and suggest that the small intestine, which produces ≈30% of plasma HDL in mice,[110] may be particularly important in regulating HDL function and systemic inflammation.[108,109,111–113]

Perspective

Although HDL-C is a very promising marker and potential therapeutic target based on its epidemiological data and the effects of healthy HDL in vitro in endothelial cells and macrophages, as well as based on infusion studies of reconstituted HDL in patients with hypercholesterolemia, it still remains uncertain whether or not HDL-C–raising drugs will become part of our preventive armamentarium in the future. Recent studies found that HDL got modified in patients with CAD or ACS because of oxidative processes and alterations in its composition (proteome and lipidome/metabolome remodelling), leading to HDL dysfunction. Therefore, it will be important to demonstrate that novel drugs not only increase HDL-C plasma levels but also improve HDL function[114] in patients at high cardiovascular risk.

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High-Density Lipoprotein: Vascular Protective Effects, Dysfunction, and Potential as Therapeutic Target

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